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Transmission of *Burkholderia cepacia* Complex: Evidence for New Epidemic Clones Infecting Cystic Fibrosis Patients in Italy

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To analyze national prevalence, genomovar distribution, and epidemiology of the *Burkholderia cepacia* complex in Italy, 225 putative *B. cepacia* complex isolates were obtained from 225 cystic fibrosis (CF) patients attending 18 CF centers. The genomovar status of these isolates was determined by a polyphasic approach, which included whole-cell protein electrophoresis and *recA* restriction fragment length polymorphism (RFLP) analysis. Two approaches were used to genotype *B. cepacia* complex isolates: BOX-PCR fingerprinting and pulsed-field gel electrophoresis (PFGE) of genomic macrorestriction fragments. A total of 208 (92%) of 225 isolates belonged to the *B. cepacia* complex, with *Burkholderia cenocepacia* as the most prevalent species (61.1%). Clones delineated by PFGE were predominantly linked to a single center; in contrast, BOX-PCR clones were composed of isolates collected either from the same center or from different CF centers and comprised multiple PFGE clusters. Three BOX-PCR clones appeared of special interest. One clone was composed of 17 *B. cenocepacia* isolates belonging to *recA* RFLP type H. These isolates were collected from six centers and represented three PFGE clusters. The presence of insertion sequence IS*1363* in all isolates and the comparison with PHDC reference isolates identified this clone as PHDC, an epidemic clone prominent in North American CF patients. The second clone included 22 isolates from eight centers and belonged to *recA* RFLP type AT. The genomovar status of strains with the latter RFLP type is not known. Most of these isolates belonged to four different PFGE clusters. Finally, a third clone comprised nine *B. pyrrocinia* isolates belonging to *recA* RFLP type Se13. They represented three PFGE clusters and were collected in three CF centers.

In the late 1970s and 1980s, reports on the recovery of *Burkholderia cepacia* from cystic fibrosis (CF) specimens began to appear (29), and the emergence of this pathogen was subsequently reviewed (25, 35, 36). Polyphasic taxonomic studies identified bacteria tentatively classified as *B. cepacia* as a complex of at least nine closely related species (genomovars). This *B. cepacia* complex consists of *B. cepacia*, *Burkholderia multivorans*, *Burkholderia cenocepacia*, *Burkholderia stabilis*, *Burkholderia vietnamiensis*, *Burkholderia dolosa*, *Burkholderia ambifaria*, *Burkholderia anthina*, and *Burkholderia pyrrocinia* (representing genomovars I through IX, respectively) (10, 11, 64–67).

From the first description of the occurrence of different genomovars in CF patients (64), it was evident that all *B. cepacia* complex species were potentially capable of human infection. Studies of the distribution of *B. cepacia* complex species in CF patients revealed a highly disproportionate distribution, with most isolates belonging to either *B. multivorans* or *B. cenocepacia*; in contrast, *B. stabilis*, *B. ambifaria*, and *B. anthina* were rarely isolated (1, 2, 37, 53, 58). Compelling evidence that *B. cepacia* complex strains can spread between CF patients either by social contacts or via hospital admission was provided by several studies (25, 33, 34, 48).

B. cepacia complex infections have a considerable impact on clinical outcome, due to the intrinsic antimicrobial resistance of these organisms and a lack of effective antibiotics. Chronic pulmonary *B. cepacia* complex infections are associated with increased rates of morbidity and mortality and in some patients are responsible for a dramatic and fatal deterioration of lung function known as cepacia syndrome (29, 33). Poor outcome in lung transplant recipients has also led some clinicians to consider these infections as a contraindication to transplantation (7, 19). Some CF patients may be transiently colonized, and others may be chronically or asymptomatically colonized, suggesting differences in host response or pathogenic potential among *B. cepacia* complex bacteria. Prevention of pulmonary infection is vital for CF patients; hence, the importance of

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stringent infection control policies, including patient segregation, has been strongly emphasized (24, 36, 49, 54, 63). However, it is also recognized that segregation places considerable social and personal burdens on CF patients and their families. To clarify how broadly such protocols should be applied, a more complete knowledge of the distribution, transmissibility, and clinical impact of *B. cepacia* complex members is urgently needed. The degree to which national and international guidelines are followed varies among microbiology laboratories (57), and misidentification of *B. cepacia* complex is common (45). Furthermore, due to the taxonomic complexity of these bacteria and the emergence of phenotypically similar species, the identification of individual genomovars is often a difficult challenge (10, 11, 43, 56, 64–66).

A better understanding of the epidemiology and pathogenic potential of *B. cepacia* complex species is vital to ensure optimal prevention and management of CF lung disease. The goal of the present study was to evaluate the epidemiology of *B. cepacia* complex bacteria infecting CF patients in Italy by using BOX-PCR analysis and pulsed-field gel electrophoresis (PFGE) of genomic macrorestriction fragments. The latter approach has a higher index of discrimination and is optimal for studying local *B. cepacia* complex epidemiology (3), whereas BOX-PCR fingerprinting is considered to be more appropriate for long-term *B. cepacia* complex epidemiology (3, 12, 15). BOX-PCR fingerprinting is a repetitive element sequence-based typing method that differentiates microorganisms by using primers complementary to interspersed repetitive consensus sequences that amplify diverse-sized DNA fragments between the repetitive BOX elements (47). While originally detected in *Streptococcus pneumoniae* (47), BOX elements with BOX-A1R as the single primer in the PCR assay were subsequently used as target for PCR-based fingerprinting in a range of different bacteria, including *B. cepacia* complex bacteria (3, 12, 15, 32).

MATERIALS AND METHODS

Bacterial strains. To analyze the prevalence, genomovar status, and epidemiology of the *B. cepacia* complex in Italy, all patients presumptively colonized by the *B. cepacia* complex attending 18 of 20 Italian CF centers from 1997 to 2003 were included in the study. A total of 225 isolates were obtained from sputum samples of 225 CF patients. All bacterial strains were isolated, following international guidelines, and were tentatively identified as members of the *B. cepacia* complex by commercial tests, including API20NE (Biomerieux, Rome, Italy) and BBL Cristal (Becton Dickinson, Milan, Italy).

All isolates were stored at −80°C. *B. cepacia* complex genomovar status was determined by a polyphasic approach, including whole-cell protein electrophoresis and *recA* restriction fragment length polymorphism (RFLP) analysis by means of the restriction enzymes HaeIII and MnlI.

SDS-PAGE of whole-cell proteins. Whole-cell protein extracts were prepared, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as previously described (51). The densitometric analysis, normalization, and interpolation of the protein profiles and numerical analyses were performed with the GelCompar software package (Applied Maths). The Pearson product moment correlation coefficient was used to calculate similarity levels between the patterns.

***recA* RFLP analysis.** RFLP analyses were performed as described previously (43). Electrophoretic separation of the restriction fragments was, however, performed by SDS-PAGE. Briefly, 10 ml of a 40% (wt/vol), 29:1 acrylamide:bisacrylamide monomer solution (National Diagnostics) was mixed with 5 ml 10× Tris-borate-EDTA (TBE) buffer and 34.5 ml sterile MilliQ Ultrapure water to obtain an 8% polyacrylamide gel. PhiX174 DNA/HinfI (Promega) comprising 20 DNA fragments ranging from 24 to 726 bp was used as a size marker. Patterns were stored and analyzed with the BioNumerics 3.5 software package (Applied Maths).

Pulsed-field gel electrophoresis analysis. Pulsed-field gel electrophoresis was performed as described by Butler et al. (5) with the following modifications. A suspension of bacteria was made in 75 mM NaCl–25 mM EDTA (pH 7.5) (SE buffer), standardized, and mixed with an equal volume of molten 1.2% low-melt agarose (Bio-Rad, Hemel Hempstead, United Kingdom). Bacteria were lysed using lysis buffer containing Triton X-100 (5 μl/ml). DNA, restricted with XbaI and SpeI (Gibco BRL, Paisley, United Kingdom) was separated by PFGE by using the CHEF DRII system (Bio-Rad) in 0.5× TBE Buffer with pulse times of 2.9 to 35.4 s at 200 V for 20 h at 14°C. Clonality was identified based on the recommendations of Tenover et al. (61).

BOX-PCR. DNA from each isolate was prepared by heating one colony at 95°C for 15 min in 20 μl of lysis buffer containing 0.25% (wt/vol) SDS and 0.05 M NaOH. Following lysis, 180 μl of distilled water was added, and the DNA solutions were stored at 4°C. Repetitive sequence-based PCR typing with a BOX-A1R primer (5′-CTACGGCAAGGCGACGCTGACG-3′) was carried as described previously (52). Briefly, 1 μl of DNA solution was mixed with 2 U of *Taq* DNA polymerase (Red Goldstar; Eurogentec), 1.25 μl of 25 mM (each) of deoxynucleoside triphosphates (Pharmacia), 2.5 μl of dimethyl sulfoxide, 0.2 μl of bovine serum albumin (10 mg/ml) (Boehringer), 5 μl of 5× Gitschier buffer (52), and 1 μl of primer (0.3 μg/ml) in a final volume of 25 μl. Amplification was carried out as follows: after initial denaturation for 7 min at 95°C, 30 amplification cycles were completed, each consisting of 1 min at 94°C, 1 min at 52°C, and 8 min at 65°C. A final extension of 16 min at 65°C was applied. PCR products were separated on a 1.5% agarose gel in 0.5× TBE buffer (55 V for 960 min at 4°C). A reference marker comprising a mixture of 100-bp and 500-bp molecular rulers (Bio-Rad) was used multiple times on each gel to allow normalization. Following staining with ethidium bromide and visualization by UV illumination, gels were analyzed with the software package BioNumerics 3.5 (Applied Maths).

Insertion sequence IS*1363* PCR. The assay for the detection of insertion sequence IS*1363* (specific for *B. cenocepacia* strains ET12 and PHDC) was performed as previously described (40). Briefly, template DNA was prepared as described above. IS*1363*-specific PCR was performed with oligonucleotide primers P1 (5′-GCTTAATAGGATGGTCAG-3′) and P2 (5′-TCCATGACCACC GTACAACTC-3′) that target sequences within IS*1363*. PCR was performed in 25-μl reaction mixtures containing 2 μl of bacterial cell lysate, 1 U of *Taq* polymerase (QIAGEN), 0.25 mM deoxynucleoside triphosphates, 0.2 μM (each) primers P1 and P2, and 2.5 μl 10× PCR buffer (QIAGEN). Amplification was carried out by using a PTC-100 programmable thermal cycler (MJ Research) under the following conditions: 95°C for 2 min; 30 cycles, each consisting of 95°C for 45 s, 55°C for 45 s, and 72°C for 45 s; and 72°C for 10 min. The resulting 235-bp amplicon was visualized after gel electrophoresis and staining with ethidium bromide. DNA from *B. cenocepacia* AU1054 was used as a positive control.

RESULTS

Species identification. Of 225 isolates, 208 isolates were confirmed as *B. cepacia* complex bacteria, representing 73.5% of Italian CF patients colonized by *B. cepacia* complex (22, 44). In 2002, the Italian Register of Cystic Fibrosis reported 3,791 living patients (52a); therefore, the overall prevalence of *B. cepacia* complex is 7.5%. To identify non-*B. cepacia* complex isolates, whole-cell protein electrophoretic profiles of all isolates were prepared and compared with a database comprising a large number of gram-negative nonfermenting bacteria that are regularly misidentified as members of the *B. cepacia* complex (10, 11, 13). Of the isolates, 10 were identified as *Burkholderia gladioli*, two as *Stenotrophomonas maltophilia*, one as *Achromobacter xylosoxidans*, one as *Herbaspirillum huttiense*, one as *Inquilinus limosus*, and two as *Pseudomonas* sp. (data not shown). The misidentified organisms represented 8% of the received isolates (17 of 225), showing the good performance of the Italian laboratories. Among the 208 isolates confirmed as *B. cepacia* complex bacteria, 13 isolates were identified as *B. multivorans* and 2 were identified as *B. vietnamiensis* by whole-cell protein electrophoresis (data not shown). As the

TABLE 1. Distribution of *B. cepacia* complex species in 18 Italian centers

B. cepacia complex species	No. of strains in center[a]:																		Total no. (%) of strains
	a	b	c	d	e	f	g	h	i	l	m	n	o	p	q	r	s	t	
B. cepacia[b]		1	3					3	4		1					3	1		16 (7.7)
B. multivorans	1		4								3			2	2	1			13 (6.3)
B. cenocepacia	7	2	30	2	4	1	18	13	1	5	3		1		7	7	20	6	127 (61.1)
B. stabilis			1					4	1		1				1	2	1	2	13 (6.3)
B. vietnamiensis								1									1		2 (1)
B. ambifaria					1														1 (0.5)
B. pyrrocinia			3						5		1								9 (4.3)
Undetermined			8				3	3	1	6	2	1	2			1			27 (13)
Total	8	3	49	2	5	1	21	24	12	11	11	1	3	2	10	14	23	8	208

[a] One strain per patient has been isolated.
[b] *B. cepacia* constitutes genomovar I.

latter technique is often not sufficiently discriminatory to differentiate isolates belonging to the remaining *B. cepacia* complex genomovars (10, 11), all *B. cepacia* complex isolates were further identified by means of *recA* RFLP analysis using HaeIII and MnlI as restriction enzymes (43). Table 1 presents the distribution of *B. cepacia* complex species among Italian centers. Sixteen isolates (7.7%) were identified as *B. cepacia* (genomovar I): 11 isolates belonged to HaeIII *recA* RFLP type E, 3 isolates belonged to HaeIII-*recA* RFLP type K, and 2 belonged to a novel HaeIII-*recA* RFLP type designated Se20. The 13 *B. multivorans* isolates (6.3%) belonged to HaeIII-*recA* RFLP types F (11 isolates) and R (2 isolates). The *B. cenocepacia* isolates represented the major fraction among the Italian isolates (61.1%) and belonged to HaeIII-*recA* RFLP types G (50 isolates), H (21 isolates), I (38 isolates), and U (18 isolates). Thirteen isolates (6.3%) belonged to *B. stabilis* HaeIII-*recA* RFLP type J. One isolate each (1%) belonged to *B. vietnamiensis* HaeIII-*recA* RFLP types A and B and to *B. ambifaria* HaeIII-*recA* RFLP type L. Nine *B. pyrrocinia* isolates (4.3%) belonged to HaeIII-*recA* RFLP type Se13. For 27 isolates (13%), the genomovar status remained undetermined, as they represented RFLP types for which the taxonomic status has not yet been clarified; 22 isolates belonged to HaeIII-*recA* RFLP type AT (10.6%), 2 (1%) belonged to HaeIII-*recA* RFLP type W, and 1 belonged to HaeIII-*recA* RFLP type S. Two isolates had well-established HaeIII-*recA* RFLP profiles (H and J) but upon digestion of the amplicon by MnlI proved to be aberrant from the typical type H and J isolates.

Strain typing. To investigate the epidemiology of *B. cepacia* complex in Italy over a 7-year period, BOX-PCR fingerprints were generated for all isolates (3, 12, 15). The reproducibility of the BOX-PCR fingerprints for isolates representing various *B. cepacia* complex species was in the range of 80 to 85%, which corresponds with results from previous studies (3, 12). In contrast with other PCR-based typing methods, the discriminatory power of BOX-PCR fingerprinting was not as high as for PFGE analysis, for instance; however, BOX-PCR profiles have been found useful for strain typing, as well as for the identification of infraspecific clusters of strains (23, 41).

For this reason, the similarity cutoff level to identify clonally related isolates was set as stringently as possible (at 80%) for all *B. cepacia* complex species (referred to below as BOX

TABLE 2. Major *B. cepacia* complex clones identified by BOX-PCR

BOX cluster	No. of isolates[a]	*HaeIII-recA* RFLP type	*B. cepacia* complex species	No. of participating centers (designation)
1	22	AT	Undetermined	8 (n, h, o, g, l, r, i, c)
2	7	E	*B. cepacia*[b]	4 (m, h, r, c)
3	12	G	*B. cenocepacia*	5 (l, t, o, e, d)
4	31	G	*B. cenocepacia*	7 (h, c, r, g, t, i, q)
5	17	H	*B. cenocepacia*	6 (c, g, q, h, s, m)
6	13	I	*B. cenocepacia*	4 (s, h, c, m)
7	14	I	*B. cenocepacia*	3 (c, h, g)
8	9	J	*B. stabilis*	6 (h, c, i, s, m, q)
9	9	Se13	*B. pyrrocinia*	3 (i, m, c)
10	17	U	*B. cenocepacia*	6 (a, r, q, g, h, b)

[a] One isolate per patient has been isolated.
[b] *B. cepacia* constitutes genomovar I.

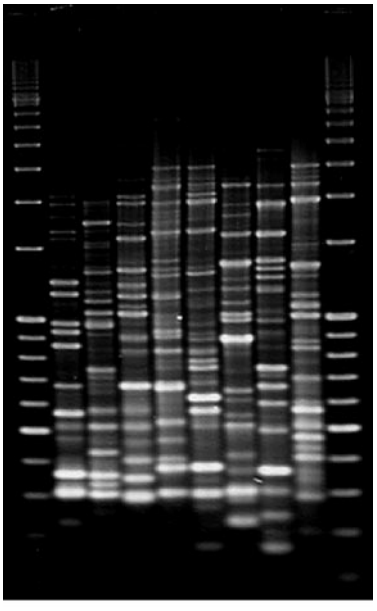

FIG. 1. BOX-PCR profiles of isolates belonging to the major *recA* RFLP types AT, E, G, H, I, J, Se13, and U (lanes 1 to 8).

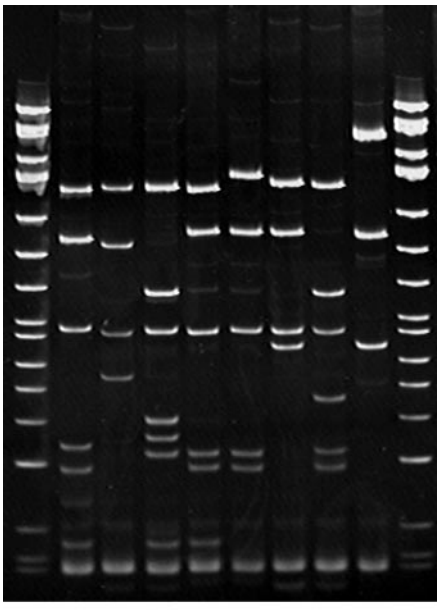

FIG. 2. HaeIII-*recA* RFLP profiles of types AT, E, G, H, I, J, Se13, and U (lanes 1 to 8).

clusters). Ten major BOX clusters, encompassing the majority (72.6%) of the isolates examined, were identified. The BOX profiles of remaining isolates were unique or represented small clusters comprising two to three isolates (data not shown). Table 2 gives an overview of the number of isolates belonging to each of the major BOX clusters, their HaeIII-*recA* RFLP type, genomovar, and CF centers involved. Figure 1 illustrates the BOX-PCR profiles of isolates belonging to the major *recA* RFLP types (AT, E, G, H, I, J, Se13, and U), while the HaeIII-*recA* RFLP profiles of the same types are shown in Fig. 2. All 208 isolates were subsequently typed by PFGE.

The criteria used to define clonality for PFGE were as described by Tenover et al. (61): profiles showing differences of up to three bands were regarded as closely related, while those showing a four- to six-band difference were considered to be possibly related, provided there was an epidemiological link between the isolates.

BOX-PCR cluster 1 (HaeIII-*recA* RFLP type AT) included 22 isolates, which were distributed among four PFGE clusters as follows. Two PFGE clusters were composed of isolates from the same center (seven and five isolates, respectively), another PFGE cluster included six isolates from four different centers, and the last PFGE cluster was composed of three isolates collected from three centers. The remaining isolate showed a unique PFGE pattern. Figure 3 shows the four different PFGE profiles produced by these isolates. The BOX-PCR clone 5 (HaeIII-*recA* RFLP type H) included 17 isolates representing three different PFGE clusters composed of 2, 3, and 5 isolates, respectively. Isolates within each of the PFGE clusters were from a single center. The remaining seven isolates all had unique PFGE profiles (data not shown). In addition, we also investigated all BOX cluster 5 isolates for the presence of IS*1363* using specific IS*1363* PCR. All BOX cluster 5 isolates contained this genetic marker (data not shown).

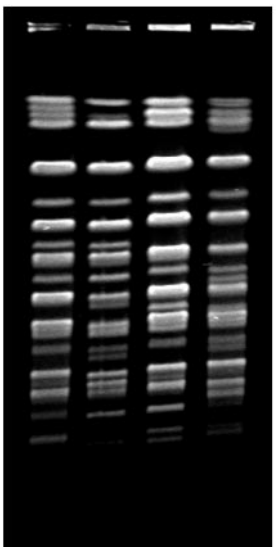

FIG. 3. PFGE profiles representing the four different clusters within the 22 isolates of BOX-PCR cluster 1 (HaeIII-*recA* RFLP type AT).

All but two (seven isolates) of BOX-PCR clone 9 (HaeIII-*recA* RFLP type Se13) isolates belonged to a single clone and were from two different centers. The remaining two isolates in this group failed to produce any PFGE profile (data not shown).

Among the remaining isolates examined, several additional center-specific PFGE clusters were identified, corroborating results obtained by BOX-PCR analysis (data not shown).

DISCUSSION

The present study reports on the epidemiology of *B. cepacia* complex bacteria infecting CF patients in Italy. Strains from 208 out of 283 (73.5%) Italian CF patients colonized by *B. cepacia* complex were collected (22, 44; data are from reference 44 and the present study). In 2002, the Italian Register of Cystic Fibrosis reported 3,791 living CF patients (52a); therefore, the overall prevalence of *B. cepacia* complex is 7.5%. Similar results have been reported from previous studies of patients in Italy (22, 60), confirming the efficacy of the segregation policies actually adopted by all Italian CF centers (22). Higher prevalences (about 20%) have been observed in some Italian centers and were associated with local spread of *B. cenocepacia* (1, 44).

Of 208 isolates collected from 208 patients attending 18 Italian CF centers, the majority (61.1%) of isolates belonged to *B. cenocepacia*, whereas 4.3 to 7.7% of isolates belonged to *B. cepacia*, *B. multivorans*, *B. stabilis*, and *B. pyrrocinia.* The other genomovars accounted for 1% or fewer of the isolates received (*B. vietnamiensis* and *B. ambifaria*) or were absent (*B. dolosa* and *B. anthina*). These data confirm and extend results from previous studies of *B. cepacia* complex bacteria infecting Italian CF patients (1, 44, 49). About 13% of the *B. cenocepacia* isolates belonged to HaeIII-*recA* RFLP type U, which was reported as a major problem in the Genoa CF center (44). The HaeIII-*recA* RFLP type U isolates of the

present study were obtained from six CF centers throughout Italy.

This predominance of *B. cenocepacia* in CF patients in Italy resembles the situation reported in Canada (80% prevalence) (58), the United States (50%) (53), the United Kingdom (76%) (9), and the Prague and Lisbon CF centers in Portugal, with prevalences of 90% and 52%, respectively (17, 21). Some national reports showed that other genomovars reach significant percentages, such as *B. cepacia* (genomovar I) in Australia (29%) (31); *B. multivorans* in France (51%) (4), the United States (38%) (37), the United Kingdom (39%) (62), and Belgium (18); or *B. stabilis* (54%) in the Slovak Republic (21). In the present study, the genomovar status of 13% of the strains was undetermined (Table 1). Isolates belonging to HaeIII-*recA* RFLP type AT accounted for most of these isolates. Isolates with the same RFLP type were recovered as contaminants from dialysis equipment in Brazil (42). BOX-PCR was chosen as the initial genotyping tool because it was previously shown that it is a useful tool to study the large-scale epidemiology of *B. cepacia* complex bacteria (3, 12). With the exception of small clusters comprising two to three isolates, 10 major BOX clusters were delineated. An analysis of the isolates by PFGE revealed that each of these BOX clusters comprised isolates that were considered unrelated by conventional criteria (61). This difference in typing resolution for *B. cepacia* complex bacteria confirmed previous reports (12). Three BOX clusters appeared of special interest. We report the first case of epidemic spread of HaeIII-*recA* RFLP type AT among CF patients (BOX cluster 1). The latter clone was isolated from 22 patients in eight CF centers (Table 2) and comprised multiple clusters of isolates, which were also considered clonal following interpretation of PFGE patterns.

The 17 BOX cluster 5 isolates were derived from patients in six CF centers (Table 2). Their BOX fingerprints were identical to those of strain PHDC (16), a well-characterized epidemic *B. cenocepacia* strain recovered from numerous CF patients and environmental samples in the United States (8, 39). The presence of IS*1363*, an insertion sequence specific for the highly epidemic strains *B. cenocepacia* ET12 (HaeIII-*recA* RFLP type G) and PHDC (HaeIII-*recA* RFLP type H) was confirmed in all BOX cluster 5 isolates. Identification as PHDC was further confirmed by comparison of random amplified polymorphic DNA profiles, as reported previously (16). Most remarkably, this epidemic clone was recognized in CF specimens from patients in several other European countries (16). When examined by PFGE, seven of these isolates represented a single PFGE cluster and several isolates with unique profiles.

Finally, BOX cluster 9 comprised nine isolates from three centers, which were identified as *B. pyrrocinia*, an organism not previously associated with spread in CF patients. When examined by PFGE, these isolates represented two PFGE clusters (specific for a single center each).

In general, the comparison between the PFGE and BOX-PCR genotyping data demonstrated that BOX clusters comprised isolates from multiple centers representing multiple PFGE clusters. In contrast, most PFGE clusters comprised isolates specific for a single center. While the latter is most likely indicative of intracenter spread through patient-to-patient contact (1, 2, 6, 44), the former is more difficult to explain. Although not common, isolates with the same PFGE profile have been observed in different Italian centers in the present study (e.g., in one of the HaeIII-*recA* RFLP type AT clones) and in a previous study in Italy (1). We consider isolates with the same BOX-PCR profile but with different PFGE profiles as examples of recent divergence from a common ancestral strain (14). Patients may become infected by such isolates through patient-to-patient transfer or through acquisition from a common source. The latter implies that such strains would be widely distributed in the environment and would have the potential to infect CF patients.

This latter hypothesis is supported by a number of observations. Govan et al. (27) provided genomic and phenotypic evidence of a clonal relationship between the *B. cepacia* type strain, isolated by W. Burkholder in the 1940s from a decaying onion, and an isolate recovered over 50 years later from the respiratory secretions of a patient with CF. In addition, LiPuma et al. (38) reported the isolation of the PHDC clone from numerous patients and agricultural soils of several years (14, 15, 38). These observations confirm that genotypically diverging *B. cepacia* complex clones can prevail in environmental sources and infect CF patients on multiple occasions. Although to our knowledge the highly transmissible *B. cenocepacia* lineage ET12 has not been isolated from environmental sources, it has been recovered from CF specimens from patients in Europe, North America, Australia, and New Zealand (1, 14, 15, 25, 26, 30, 50, 59) with minor differences in genotype and phenotypic properties (50). It seems unlikely that this global distribution can be explained through patient-to-patient contacts alone. Interestingly, similar evidence is emerging for other CF pathogens as well. Genotypically diverse isolates of *P. aeruginosa* clone C have been recovered from CF, non-CF, and environmental specimens throughout Europe (20); since the early 1990s, European-wide clones of *Staphylococcus aureus* have been isolated from CF and non-CF sources without epidemiological relationships (55).

The present study provides an overview of the prevalence and distribution of *B. cepacia* complex species and strains in Italy and a comparison with other countries. It confirms that the epidemiology of the *B. cepacia* complex bacteria in Italy is mainly characterized by the acquisition and spread of *B. cenocepacia* strains and presents the first evidence of spread of *B. pyrrocinia* and of a strain belonging to HaeIII-*recA* RFLP type AT. Furthermore, our study confirms the presence of PHDC, an epidemic lineage of *B. cenocepacia*, in patients from multiple CF centers in Italy. These findings substantiate previous reports of the spread of non-*B. cenocepacia B. cepacia* complex species including *B. cepacia* (genomovar I) (3), *B. multivorans* (28, 46, 68), and *B. dolosa* (3). Finally, the occurrence of isolates with distinct PFGE profiles but with the same BOX-PCR profiles suggests the nationwide environmental distribution of genetically diverging clones.

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